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Effect of *Phyllanthus niruri* leaves extract on urinary stone forming minerals

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Abstract-Faced with the growing evidence that stone disease is of recurrent in nature, variety of substances have been used in an effort to dissolve renal stones and to prevent recurrence. In modern medicine, allopurinol and D-penicillamine are the effective drugs for dissolution and control of recurrence of uric acid and cysteine calculus, but we hardly have any effective and useful drug for the treatment of calcium oxalate, calcium phosphate and mixed type of stones. In present work we are reporting the inhibition efficiency of *Phyllanthus niruri* leaves extract towards the mineralisation and demineralisation of calcium oxalate, calcium phosphate and calcium carbonate. We have also estimated the phosphate content of the above natural product extract. *Phyllanthus niruri* popularly known as "Stone-breaker" is a plant belonging to Euphorbiaceae family with a worldwide distribution. More than 50 compounds were identified in *Phyllanthus niruri*, including alkaloids flavanoids, lignans and triterpenes. Interestingly, given that the maintenance of normal levels of calcium is critical to the function of many plants including plant rigidity, protection, detoxification (heavy metals or oxalic acid), ion balance and even light reflection and because high cellular free calcium concentration is dangerous for these organising higher plant developed very efficient way to neutralise Ca^{2+} ions, by forming complex with oxalate.

Keywords : recurrent, calculus, mineralisation, detoxification

INTRODUCTION

Urinary stones affect 10-12% of population in industrialized countries. Their incidence has been increasing over the last years and the age of onset is decreasing. In addition, the recurrence rate is high, more than 50% after 10 years. Given that urine is naturally a super saturated solution crystalluria is often observed in normal individuals, but if crystals remain apart from each other, they are washed away by the urine flow. However under certain circumstances they bind each other due to chemical and electrical forces triggering the process of aggregation. The crystals or aggregates then attach to the epithelium, which allow them to grow further and form stone.

Alternative treatments such as the traditional herbal treatments can complement pharmacotherapies for prevention and/ or treatment of urolithiasis with less expense and fewer side effects. We have been evaluating the effects of *Phyllanthus niruri* on several stone forming minerals. Experiments studies performed by our group and others have produced interesting and hopeful data

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concerning the potential therapeutic use of *Phyllanthus niruri* to treat and/or prevent stone formation.

EXPERIMENT

Preparation of *Phyllanthus niruri* leaves extract and its acid hydrolysate

100 g of *Phyllanthus niruri* leaves was crushed and the juice was centrifuged for a few minutes and again filtered through G4 crucible. The clear filtrate obtained was used for assaying its inhibitory effect on calcium oxalate, calcium phosphate and calcium carbonate.

Now to prepare the hydrolysed extract, the filtrate was treated with about 10 ml of 2N HCl and refluxed for 2 hours. After cooling the hydrolysed extract, solid sodium bicarbonate was added with constant stirring to bring the pH back to approximately 7 and filtered through G4 crucible.

Dissolution of calcium oxalate, calcium phosphate and calcium carbonate

Known weights of calcium oxalate, calcium phosphate and calcium carbonate were suspended separately in 100 ml *Phyllanthus niruri* leaves extract (fresh/hydrolysed). The extracts were prepared in manner mentioned before. The suspension was stirred constantly for an hour on a magnetic stirrer at room temperature and then filtered through G3 crucible. The precipitate, that is undissolved salt was washed with distilled water, dried and weighted out. By subtracting the weight of undissolved salt from the initial weight taken, we get the amount of dissolved salt (Table-1).

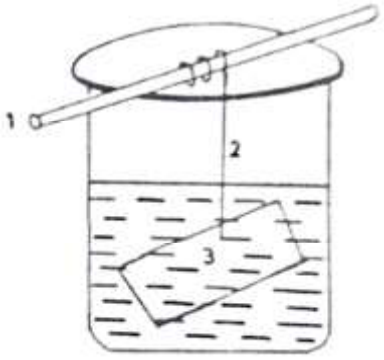
Inhibitory effect of *Phyllanthus niruri* leaves extract (fresh/hydrolysed) on the mineralisation of calcium oxalate, calcium phosphate and calcium carbonate.

The model system used to study the inhibitory effect of *Phyllanthus niruri* leaves extract (fresh/hydrolysed) on the mineralisation of calcium oxalate, calcium phosphate and calcium carbonate has some resemblance to the one (model) used by Kabra *et al.* Model consisted of two beakers of 100 ml capacity. In one of them 70 ml of 0.01 M solution of $CaCl_2$, was placed; to it 20 ml of *Phyllanthus niruri* leaves extract (fresh/hydrolysed) inhibitor was added. In the second beaker 70 ml of 0.01 M solution of sodium oxalate ($Na_2C_2O_4$) /sodium phosphate (Na_2PO_4) / sodium carbonate (Na_2CO_3) was placed and as above, 20 ml *Phyllanthus niruri* leaves extract (fresh/hydrolysed) was added to it. Two filter papers (whatman no 41,11 cm) were folded in square shape, so that both the wicks, were then suspended separately into the solution of the two beakers. Suspension was done with the help of copper wires and glass rods as shown in Fig - 1.

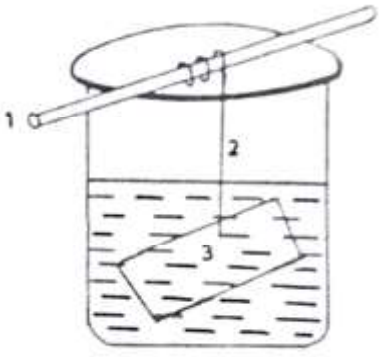
This set of two beakers was termed 'EXPERIMENTAL SET'. Next a similar assembly of two beakers with suspended filter paper wicks (weighed and noted separately) were arranged at the side of the experimental set. In this case, however, the contents of the beakers were a little different from the one in the experimental set. One of the beakers of this set contained 90 ml of 0.01 M solution of $CaCl_2$ and the other beaker contained 90 ml of 0.01 M $Na_2C_2O_4$/Na_3PO_4/Na_2CO_3 solution. No inhibitor was added in this case. This set was termed 'BLANK SET'.

APPARATUS SET-UP

1. Glass Rod 2. Copper wire 3. Wick

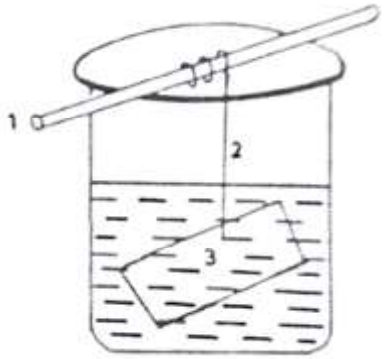


70 ml of $CaCl_2$ Solution + 20 ml of distilled water

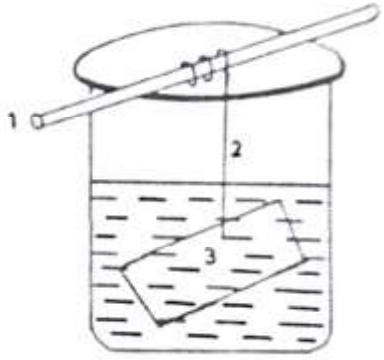


70 ml of $Na_2C_2O_4$/ Na_3PO_4/Na_2CO_3 Solution+20ml of distilled water

Blank Set



70 ml of $CaCl_2$ Solution + 20 ml of inhibitor



70 ml of $Na_2C_2O_4$/ Na_3PO_4/ Na_2CO_3 Solution + 20ml of inhibitor

Experimental Set

Fig-1

Next, the two filter paper wicks suspending in the two beakers of 'Experimental set' were interchanged at the end of every five minutes interval. This meant that the filter paper wick suspending in $CaCl_2$ solution were transferred to $Na_2C_2O_4$/ Na_3PO_4/ Na_2CO_3. solution. This interchange of wicks at the end of every five minutes was continued for four hours.

Similarly, the filter paper wicks suspending in the beakers of 'BLANK SET' were also interchanged at the end of every five minutes for four hours. Work was carried out at room temperature. At the end of the experimentation, the filter paper wicks of 'experimental set' as well as blank set after washing, were dried at 90°C in an air oven, cooled to room temperature and weighed separately. From these weights of filter papers, their corresponding weights, noted from experimentation, were deducted and thus the amount of crystalloid ($Ca_2C_2O_4$/$Ca_3(PO_4)_2$/$CaCO_3$) deposited on each paper was determined. Average deposition of oxalate in the experimental set was calculated by adding the deposition on both the papers (of experimental set) and dividing it by two. Similarly average deposition on the blank set papers was also calculated out. Further the difference between the average deposition of oxalate/ phosphate/ carbonate in blank and experimental set was calculated; this was termed 'net inhibition'. This net inhibition value was divided by the average amount deposited in the blank set and multiplied by 100 to get 'percentage inhibition' value.

Similar 'Experimental' and Blank' sets under the experimental conditions were run for different concentrations, viz. M/100, M/20. Inhibitor concentration was however kept the same in all the runs (Table -2, Table-3, Table - 4).

$$\text{Net Inhibition} = \frac{\text{Average deposition of } C_2O_7^{2-}/PO_4^{3-}/CO_3^{2-} \text{ in experimental set} - \text{Average deposition of } C_2O_7^{2-}/PO_4^{3-}/CO_3^{2-} \text{ in blank set}}{\text{Average deposition of } C_2O_7^{2-}/PO_4^{3-}/CO_3^{2-} \text{ in blank set}} \times 100$$

RESULT

Table 1.(a)- Solubilities of calcium phosphate, calcium oxalate and calcium carbonate in *Phyllanthus niruri* leaves extract.

Sl.no.	Natural Product Extract	Solubility mg/100 ml of extract		
		Calcium Oxalate	Calcium Phosphate	Calcium Carbonate
01.	*Phyllanthus niruri* leaves extract (fresh)	17.40	14.07	20.8
02.	*Phyllanthus niruri* leaves extract (hydrolysed)	21.64	18.50	17.5

Table 1.(b)- Inhibitory effect of *Phyllanthus niruri* extract on mineralisation of calcium oxalate/ calcium phosphate/ calcium carbonate

i.	**Contents of beaker in mineralisation of calcium oxalate**		
Experiment Set		Beaker -I	70 ml $CaCl_2$ + 20 ml inhibitor
		Beaker –II	70 ml $Na_2C_2O_4$ + 20 ml inhibitor
Blank set		Beaker –I	90 ml $CaCl_2$ solution
		Beaker –II	90 ml $Na_2C_2O_4$ solution
ii.	**Contents of beaker in mineralisation of calcium oxalate**		
Experiment Set		Beaker –I	70 ml $CaCl_2$ solution+ 20 ml inhibitor
		Beaker –II	70 ml Na_3PO_4 solution + 20 ml inhibitor solution
Blank set		Beaker –I	90 ml $CaCl_2$ solution
		Beaker –II	90 ml Na_3PO_4 solution
iii.	**Contents of beaker in mineralisation of calcium oxalate**		
Experiment Set		Beaker –I	70 ml $CaCl_2$ solution+ 20 ml inhibitor
		Beaker –II	70 ml Na_2CO_3 solution + 20 ml inhibitor solution
Blank set		Beaker –I	90 ml $CaCl_2$ solution
		Beaker -II	90 ml Na_2CO_3 solution

Table 2- Inhibitory values of *Phyllanthus niruri* extract (fresh/hydrolysed) on mineralisation of calcium oxalate. pH range- 5-7

No. of observation	Inhibitor	Strength of Salt forming solution	Accretion of Ca oxalate		Net inhibition difference (mg)	Percentage inhibition
			Blank Set	Experimental set		
1.	*Phyllanthus niruri* leaves extract (fresh)	0.01 M	567.0	449.0	98.0	17.28
2.	*Phyllanthus niruri* leaves extract (fresh)	0.05 M	396.0	290.0	106.0	26.76
3.	*Phyllanthus niruri* leaves extract (hydro)	0.01 M	578.6	415.0	127.6	22.05
4.	*Phyllanthus niruri* leaves extract (hydro)	0.05 M	412.0	193.0	219.0	29.36

Table 3- Inhibitory values of *Phyllanthus niruri* extract (fresh/hydrolysed) on mineralisation of calcium phosphate. pH range- 5-7

No. of observation	Inhibitor	Strength of Salt forming solution	Accretion of Ca oxalate		Net inhibition difference (mg)	Percentage inhibition
			Blank Set	Experimental set		
1.	*Phyllanthus niruri* leaves extract (fresh)	0.01 M	47.8	43.4	4.4	10.38
2.	*Phyllanthus niruri* leaves extract (fresh)	0.05 M	39.6	33.3	6.3	15.9
3.	*Phyllanthus niruri* leaves extract (hydro)	0.01 M	49.0	42.8	6.2	12.65
4.	*Phyllanthus niruri* leaves extract (hydro)	0.05 M	40.4	32.0	8.4	20.79

Table 4- Inhibitory values of *Phyllanthus niruri* leaves extract (fresh/ hydrolysed) on mineralisation of calcium carbonate.pH range- 5-7

No. of observation	Inhibitor	Strength of Salt forming solution	Accretion of Ca oxalate		Net inhibition difference (mg)	Percentage inhibition
			Blank Set	Experimental set		
1.	*Phyllanthus niruri* leaves extract (fresh)	0.01 M	32.0	28.0	4.0	12.5
2.	*Phyllanthus niruri* leaves extract (fresh)	0.05 M	15.0	14.0	1.0	6.6
3.	*Phyllanthus niruri* leaves extract (hydro)	0.01 M	27.6	17.8	9.8	35.5
4.	*Phyllanthus niruri* leaves extract (hydro)	0.05 M	8.2	6.7	1.5	18.3

DISCUSSION

The present study gives support to the importance of natural products containing acids and / or phosphates in the dissolution of renal calculi. To find out the exact nature of acids (i.e. whether hydroxy, amino or heterocyclic) and the type of phosphates (i.e. whether ortho, pyro, meta) is a difficult proposition, nevertheless, some approximation has been attempted. A glance at the percentage of phosphate in the natural product extracts suggests that the acid hydrolysis improves the phosphate extent. It might be due to the cleavage of more and more pd units of the proteins and their liberation of free chain phospho units. The consumed natural products most likely get hydrolysed in the digestive system.

Hydrolysates might be metabolites and these metabolites, enriched in chain phosphate content, must essentially act upon the urinary calcium (calcium dissolved oxalate/ calcium phosphate/ calcium carbonate) and keep them in state. The exact mechanism behind the dissolution/ inhibition activity of acids and pyrophosphates might be the aqueous soluble complexation; most likely the complexation by non-replacement type of chemical reactions.

Our findings in the present work suggest that the inhibitory action of *Phyllanthus niruri* leaves extracts in the mineralization and demineralization of urinary calcium (calcium oxalate/ calcium phosphate/ calcium carbonate) to be appreciable. A glance at the result shows that in the inhibition experiments, the accretion of calcium oxalate, calcium phosphate and calcium carbonate in the experimental sets is appreciably less than that in corresponding blank sets. Presence of inhibitor (i.e. the above mentioned extracts) in experimental set is responsible for the inhibition of calcium oxalate, calcium phosphate and calcium carbonate accretion. The percentage inhibition increases as the dilution of the salt forming solution increases. The percentage inhibitions are more in acid hydrolysate extract compared to their unhydrolysed extract. This is perhaps due to increased polyphosphate content in acid hydrolysate extracts. In the digestive system, where the dilution of salt forming solution would be very high, the inhibition effect of the above extracts can also be expected to be very high.

In the present work we have studied the effect of the inhibitors (natural product) in a diluted state, so as to obtain some information on their physiochemical effects although it is difficult to transfer directly the findings to the situation *in-vivo* because there might be difference between findings *in-vitro* and effects *in-vitro*. However, it can be assumed that factors affecting the inhibitor in their diluted state will also affect them in a concentrated state.

The order of solubilising capacities of extracts is more in calcium oxalate followed by calcium phosphate and calcium carbonate.

CONCLUSION

Owing to physiological toxicity of various synthetic compounds that can dissolve renal calculi of the types calcium oxalate calcium phosphate and calcium carbonate,

it is more beneficial to look into some physiologically non-toxic natural products that can dissolve calcium containing stones. With such a view in mind, we have focused our attention on some natural products viz *Phyllanthus niruri* leaves extract in the present work.

Our present findings with above natural product suggest that this is a good solubiliser of calcium oxalate, calcium phosphate and calcium carbonate. They do so by virtue of pyrophosphate and acids present in the extracts of the natural product.

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